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ATTORNEY'S DOCKET NO.
Forssmann et al PCT

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)
09/242254

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP97/04396	Aug. 13, 1997	8/13/96; 6/16/97

TITLE OF INVENTION
A METHOD FOR DETECTING THE CONDITION OF AN ORGANISM THROUGH THE MEASUREMENT OF PEPTIDES

APPLICANT(S) FOR DE/EO/US
WOLF-GEORG FORSSMANN ET AL

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. X This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ___ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. X This is an express request to begin national examination procedures (35 U.S.C. 371 (f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ___ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. X A copy of the International Application as filed (35 U.S.C. 371(c)(2)
   a. X is transmitted herewith (required only if not transmitted by the International Bureau)
   b. ___ has been transmitted by the International Bureau.
   c. ___ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. X A translation of the International Application into English (35 U.S.C.371(C)(2)
7. ___ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. ___ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ___ have been transmitted by the International Bureau.
   c. ___ have not been made; however, the time limit for making such amendments has **NOT** expired.
   d. ___ have not been made and will not be made.
8. ___ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ___ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ___ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. X An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ___ Assignment document for recording. Separate cover sheet in compliance with 37 CFR 3.28 and 3.31 included.
13. X A **FIRST** preliminary amendment
    ___ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. ___ A substitute specification
15. ___ A change of power of attorney and/or address letter.
16. X Other items or information:

    Forms 210; 409

APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO. PCT/EP97/04396	ATTORNEY'S DOCKET NO. FORSSMANN ET AL

17. X The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Search Report has been prepared by the EPO or JPO.....**$840.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.492) **$670.00**		
Neither international preliminary examination fee paid (37 CFR 1.82) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....**$ 970.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 840.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ___ 20 ___ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	13 - 20 =	0	X **$18.00**	$	
Independent Claims	1 - 3 =	0	X **$78.00**	$	
Multiple dependent claim(s) (if applicable)			+ **$260.00**	$	
TOTAL OF ABOVE CALCULATIONS =				$ 840.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				$	
SUBTOTAL =				$	
Processing fee of **$130.00** for furnishing the English translation later than ___ 20 ___ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$	
TOTAL NATIONAL FEE =				$ 840.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$ 840.00	
				Amount to be: refunded	$
				charged	$

a. X A check in the amount of $ 840.00 to cover the above fees is enclosed.

b. ___ Please charge my Deposit Account No. 03-2468 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. X The Commissioner is hereby authorized to charge any additional fees which maybe required, or credit any overpayment, to Deposit Account No. 03-2468. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Signature

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Edward R. Freedman, Reg. No. 26,048

Express Mail EL 264 529 683 US

Date of February 12, 1999

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10, on the date indicated above, and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231

Maureen E. Bitz M. Bitz

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT OR PATENTEE: Wolf-Georg FORSSMANN ET AL
SERIAL OR PATENT NO.: PCT/EP97/04396
FILED OR ISSUED: Aug. 13, 1997
GROUP:
TITLE: A METHOD FOR DETECTING THE CONDITION OF AN ORGANISM THROUGH THE MEASUREMENT OF PEPTIDES

SMALL ENTITY DECLARATION

[ ] FOR INDEPENDENT INVENTOR(S)

As a below-named inventor, I hereby declare that I am an independent inventor who (1) has not assigned, granted, conveyed, or licensed, and (2) is under no obligation under contract or law, to assign, grant, convey, or license, any rights in the invention, to any person who could not likewise be classified as an independent inventor if that person had made the invention, or to any concern which would not qualify as a small business concern or a nonprofit organization, as defined in 37 CFR 1.9(e).

[X] FOR SMALL BUSINESS CONCERN

I hereby declare that BIOVISION GmbH & Co. KG is a business concern which qualifies as a small business concern as defined in §1.9(d) - namely, (1) whose number of employees, including those of its affiliates, does not exceed 500 persons; and (2) which has not assigned, granted, conveyed, or licensed, and is under no obligation under contract or law to assign, grant, convey, or license, any rights in the invention to any person who could not be classified as an independent inventor if that person had made the invention, or to any concern which would not qualify as a small business concern or a nonprofit organization under this section; and that the exclusive rights to the invention have been conveyed to and remain with the above-identified small business concern.

I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful, false statements and the like, so made, are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the patent application or any patent issuing thereon.

Each of the undersigned hereby grants the firm of COLLARD & ROE, P.C., 1077 Northern Boulevard, Roslyn, New York 11576, U.S.A., the power to insert in this Small Entity Declaration any further identification which may be necessary or desirable to comply with the rules of the U.S. Patent and Trademark Office for filing and acceptance of this Declaration.

INVENTOR(S)

Name:
Date:

Name:
Date:

SMALL BUSINESS CONCERN:

×By Peter Schuld Knappe
×Name: Peter Schuld Knappe
×Title: Chief executive Officer
×Date: 15/04/1999

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: WOLF-GEORG FORSSMANN ET AL

PCT NO.: PCT/EP97/04396

PCT FILED: August 13, 1997 PRIORITIES: 8/13/96; 6/16/97

TITLE: A METHOD FOR DETECTING THE CONDITION OF AN ORGANISM THROUGH THE MEASUREMENT OF PEPTIDES

PRELIMINARY AMENDMENT

BOX PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Preliminary to examination, please amend this application as follows: --

IN THE CLAIMS:

Please amend claim 1 as follows: --

1 (amended). A method for detecting the condition of an organism through the measurement of peptides from a sample of said organism containing high- and low-molecular weight peptides, as an indication of the condition of said organism, without the need to recur to hypotheses, wherein

- low-molecular weight peptides are directly detected and characterized; and

- related to a reference. --

Claim 3, line 1, change "claims 1 and/or 2" to --claim 1--.

Claim 5, line 1, change "claims 3 and/or 4" to --claim 3--.

Claim 6, line 1, change "at least ... to 5" to --claim 1--.

Claim 7, line 1, change "at least ... to 6" to --claim 1--.

Claim 8, line 1, change "at least ... to 7" to --claim 1--.

Claim 9, line 1, change "at least ... to 8" to --claim 1--.

Claim 10, line 1, change "at least ... to 9" to --claim 1--.

Claim 11, line 1, change "at least ... to 10" to --claim 1--.

Claim 12, line 1, change "at least ... to 11" to --claim 1--.

Claim 13, line 1, change "at least ... to 11" to --claim 1--.

REMARKS

By this Preliminary Amendment, the multiple dependency of some of the claims has been removed so as to avoid the surcharge associated therewith. Claim 1 has been amended by inserting "without the need to recur to hypotheses". This amendment corresponds to the amendment in the international application.

Respectfully submitted,

WOLF-GEORG FORSSMANN ET AL

By _____

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ERF:im

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: ASSISTANT COMMISSIONER OF PATENTS, Washington, D.C. 20231, on February 12, 1999.

_____
M. Bitz

A method for detecting the condition of an organism through the measurement of peptides

The present invention relates to a method for detecting the condition of an organism through the measurement of peptides from a sample of said organism.

Various analytical methods are employed for detecting the condition of an organism. Thus, for example, in the diagnostics of higher organisms, when pathological results are obtained, attempts are made to fathom the causes of the pathological change on the basis of the symptoms in order to develop a causal therapy. Further, efforts are being made to develop a reference of an average "healthy" organism by sequencing the genomes of organisms and establishing "wild type genomes" in order to be able to discover individual deviations which could indicate possible pathogenic developments by performing corresponding gene analyses. A drawback of the first methodological approach is that diagnostics free from hypotheses (bias-free) cannot be performed since the diagnostics therein are already based on assumptions. A drawback of the second method is that it will not be possible for a long time to diagnose the important or even all diseases attributed to genetic dysfunctions. Another drawback of the latter method may also be that a mutation on a gene does not necessarily result in expression of the related phenotype.

Thus, it would be desirable to provide a universally employable diagnostic method by which it is possible to avoid the drawbacks mentioned and, in particular, to perform diagnostics free

from hypotheses. In addition, the diagnostic method should be universally employable, not be restricted to higher developed systems, but also be employable for detecting the condition of lower organisms. In addition, it should be easy to establish and capable of being carried out with per se known techniques.

Thus, it has been the object of the present invention to provide such a method.

Surprisingly, the object of the invention is achieved in a simple manner by a method with the features of claim 1. The subclaims pertain to preferred embodiments of the method according to the invention.

The method according to the invention for detecting the condition of an organism starts by taking a sample from the organism to be examined. This sample may also be the complete organism. The sample must contain low-molecular weight peptides, but there is no interference from high-molecular weight peptides or proteins which are also contained in the sample in addition to low-molecular weight peptides. According to the invention, the low-molecular weight peptides are directly detected and characterized and serve as indicators of the condition of the organism. It is possible to detect single peptides directly by a measuring technique, to detect several peptides by a measuring technique, or even all the low-molecular weight peptides present in the sample which can be detected by a measuring technique. Unlike conventional analytical or diagnostic methods, such as gel electrophoresis or two-dimensional electrophoresis and, for example, clinical diagnostic methods, the method according to the invention does not examine the high-molecular weight structures, such as proteins. As opposed to per se known diagnostic methods, such as radioimmunoassay or other competitive assays for the measurement of peptide hormones and the like, the low-molecular weight peptides are directly detected according to the invention by some measuring technique rather

than indirectly as in the methods mentioned. The distribution of low-molecular weight peptides in a representative cross-section of defined controls is used as a reference.

In the method according to the invention, the sample to be examined may be derived from tissue or fluid samples from the organism the condition of which is to be detected, or it may be the organism itself or parts thereof. When lower organisms are examined, the organism itself is preferably used as the sample. Such lower organisms include, in particular, single-celled organisms, such as procaryotic systems or simple eucaryotic systems, such as yeasts or other microorganisms.

According to the invention, the low-molecular peptides employed for measurement shall preferably have a molecular weight of not more than 30,000 Dalton. The lower limit is not actually critical, but dipeptides represent the lower limit of low-molecular weight peptides to be detected according to the invention. Particularly preferred are molecular weights of the low-molecular weight peptides of from 100 to 10,000 Dalton.

If required, for example, due to a changed measuring arrangement, it may be advantageous to remove high-molecular weight peptides or proteins and other biopolymers which might interfere with the measurement from the sample. This is not required, in particular, in cases where the higher-molecular weight peptide compounds are not covered by the measuring method to be employed according to the invention.

Preferably, according to the invention, mass spectroscopy is employed for detecting the low-molecular weight peptides. Particularly preferred is the so-called MALDI method (matrix assisted laser desorption ionization mass spectroscopy). If mass spectroscopy is employed as a method, it is recommendable to employ the data obtainable by said mass spectroscopy for characterizing the low-molecular weight peptides, such as their

molecular weights. It is also possible, under particular circumstances, to analyze other parameters, such as the charge of the peptides, or the characteristic retention times on chromatographic columns, or a fragment pattern of the low-molecular weight peptides, or combinations of the mass and charge of the low-molecular weight peptides.

Depending on the additional questions connected with the detection of the condition of the organism, it may be advantageous to divide the sample into several fractions and to analyze the samples under different aspects or with different measuring arrangements, and thus to detect a condition of the organism.

The organisms include, in particular, procaryotes, eucaryotes, multicellular organisms, cells from tissue cultures, cells from animals and humans. Thus, it becomes possible according to the invention to examine the condition of genetically engineered or transformed and/or conditioned organisms. This may be advantageous, in particular, for checking transformed systems in order to recognize any unexpected or undesirable properties which might have been developed by transformed organisms, for instance, by forming peptides indicative of undesirable or unexpected properties, such as toxic properties.

In particular, any deliberately or unintentionally performed manipulation (conditioning) of an organism may influence its condition, whether during the administration of medicaments, gene therapy, infections, in the working place from contact with chemical substances, in test animals, especially transgenic animals and knock-out mutants. Especially in the case of such methods, an intra- and interindividual comparison, for example, through the chronological taking of samples from an organism prior to and in the course of one of the above mentioned measures, or a comparison with untreated control organism may be used to check whether the predicted and desired changes in condition have actually occurred, and whether, in

addition or instead, unpredicted, undesirable or desirable, changes have occurred which are detected by the method according to the invention without the need to recur to hypotheses.

Therefore, the method according to the invention is also useful, for example, for accompanying clinical studies, toxicological examinations in the testing of medicaments of all kinds, for analyzing/detecting decomposition products, for the identification of gene products.

In veterinary and human medicine, the method according to the invention gains its outstanding importance by the fact that it enables the detection of the condition of the respective organism without the need to recur to hypotheses. Thus, rather than performing a confirmation assay based upon a preconceived opinion, a real overall picture of the condition of the organism examined can be created. The method according to the invention, which may be designated as a differential peptide display, is based on the fact that a particular peptide pattern is present in a healthy organism which is therefore capable of serving as a reference standard. Now, if the peptide condition of an individual is recorded and compared to that of the reference, deviations can be detected which provide a first indication of a possibly pathogenic condition. By detecting the deviations established by comparison with similar pathogenic conditions from corresponding samples of a diseased, it is then possible to identify the respective disease directly from the analysis by a mere comparison of the deviations in the peptide pattern of the sample of said individual, and correspondence of the deviation with an assigned clinical picture.

According to the invention, one may proceed as follows, in particular. Ultrafiltrates from body fluids and tissue extracts may first be used for preparing a reference sample. Recovery of the filtrate peptides and their separation into fractions is performed, for example, by collecting low-molecular weight

peptide fractions. The characterization of the peptide fractions may be effected, for example, by their retention behavior and molecular weight, which can be determined by chromatography or mass spectroscopy. For example, if an ultrafiltrate from patients suffering from a known disease is used and compared with the previously established spectrum of healthy reference subjects, the deviating pattern enables an assignment of the specific disease to the condition of the respective peptide mixture. Thus, this method may also be employed in a per se conventional manner, for example, by immediately interrogating the appropriate peptide pattern indicative of pathogenic changes. In some cases, this may even be one peptide characteristic of the respective disease. For example, if a sample is analyzed from a patient for whom a particular clinical picture can be recognized and a hypothesis for the cause of such disease exists, this specific peptide may also be interrogated in the analysis according to the invention, and if the result is positive, appropriate therapeutic schemes may be established. Thus, it is altogether possible to first take a sample from the patient, to record a condition by the method according to the invention, and then, if the presence of a deviation indicative of pathogenic conditions is established, either to perform a control measurement by per se known confirmation assays recurring to the usual clinical assays, or to perform such control measurement by specifically screening for the indicator of the pathogenic condition.

Peptides may be recovered by methods known to those skilled in the art, such as ultrafiltration of the respective starting material. When doing so, filters are used having a molecular exclusion size within the range claimed according to the invention, i.e., between that of a dipeptide and a maximum of 30,000 Dalton. By appropriately selecting the respective membranes, it is also possible to obtain fractions of particular molecular weights. Preferably, from 0.2 ml to 50 l of filtrate is obtained from the filtration, which is adjusted, for example, to

a pH value of from 2 to 4 by acidification with diluted hydrochlorid acid immediately after the end of the filtration. The amounts mentioned especially serve to examine pooled samples, for developing reference samples from healthy subjects, or for determining disease-specific peptide markers for establishing a peptide data base.

The peptides present in the filtrate after ultrafiltration are recovered by adsorption to chromatographic materials, especially cation exchangers, such as Fractogel, anion exchangers-Fractogel TMAE, and reversed phase (RP) materials, followed by elution with linear gradients or step gradients. For further purification, other chromatographic separations, especially through reversed phase materials, may optionally be effected.

The measurement of the peptide fractions is preferably performed by mass-spectrometrical analysis, especially with MALDI MS (matrix assisted laser desorption ionization mass spectrometry) or ESI MS (electrospray ionization MS). These are methods which can be used for the analysis of peptides. This preferably involves the on-line coupling of Microbore reversed phase separation and mass spectrometry (LC-MS coupling). From the data obtained, a multidimensional table is established based on retention behavior, molecular weight and signal intensity as the preferred guiding parameters. However, other quantities which can be determined with the mentioned methods may also be recorded.

The data about patients with a known basic disease obtained from the above mentioned steps are compared to the similarly obtained data from a healthy reference population. Both qualitative changes (e.g., the occurrence of new peptides or the lacking of peptides) and quantitative changes (the increased or decreased occurrence of individual peptides) are detected. If required, the targets defined by the comparative analysis may further be purified and identified by methods of peptide chem-

istry known to those skilled in the art. The sequence information obtained can then be compared with protein and nucleic acid data bases and subsequently with data from the literature. The relevance of the represented peptides with respect to the examined disease is checked by functional studies and by screenings with appropriate groups of patients.

Example 1

Use of body fluids: blood filtrate (hemofiltrate, HF)

1. Recovery of HF

HF is recovered by arterio-venous or veno-venous hemofiltration performed by techniques known to those skilled in the art with selected patients or subjects. The recovery of HF is effected in the same way, in principle, as performed as a matter of routine in patients with chronical renal disease. Through an arterial drain and venous feed (arterio-venous hemofiltration) or venous drain and venous feed (veno-venous hemofiltration), the patient's blood is passed with the aid of a hemofiltration device (e.g., Hemoprozessor, Sartorius, Göttingen; AK 10 HFM, Gambro, Hechingen) through a hemofilter (e.g., Hemoflow F 60 or Hemoflow HF 80 S, Fresenius, Bad Homburg; Hemoflow FH 77 H and Hemoflow HF 88 H, Gambro) which has a molecular exclusion size of up to 30 kDa. The filtrate volume withdrawn from the patient is substituted by an electrolyte solution (e.g., SH 01, SH 05, SH 22, SH 29, Schiwa, Glandorf).

According to the present method, a diagnostic hemofiltration is performed with the aim to obtain from 1 to 30 l of HF from a patient in the course of one hemofiltration. For avoiding proteolysis, the hemofiltrate is immediately adjusted to a pH value between 2 and 4 with diluted acid (e.g., 1 M HCl), and cooled to 4 °C.

2. Recovery of the HF peptides and separation into fractions

2.1 Peptide extraction with stepwise elution

10 l of hemofiltrate is diluted with deionized water to provide a conductivity of 6 mS/cm, and its pH value is adjusted to 2.7 with hydrochlorid acid. The HF is then applied to a chromatographic column. After binding of the HF peptides, the bound peptides are eluted with a pH step elution using 7 buffers with increasing pH values.

Chromatographic conditions:

flow for application: 100 ml/min
flow for elution: 30 ml/min
detection: 214, 280 nm
column: Vantage (Amicon, Witten), 6 cm diameter x 7 cm filling height
column material: Fraktogel TSK SP 650 M (Merck, Darmstadt)
equipment: BioCAD 250, Perseptive Biosystems, Wiesbaden-Nordenstadt

buffer	pH value	buffer substances	molarity
elution buffer 1	3.6	citric acid	0.1
elution buffer 2	4.5	acetic acid	0.1
elution buffer 3	5.0	malic acid	0.1
elution buffer 4	5.6	succinic acid	0.1
elution buffer 5	6.6	sodium dihydrogenphosphate	0.1
elution buffer 6	7.4	disodium hydrogenphosphate	0.1
elution buffer 7	9.0	ammonium carbonate	0.1

Eluates 1-7 are separately collected.

2.2 Second chromatographic separation

Eluates 1-7 are separately subjected to chromatography through a reversed phase column.

Chromatographic conditions:

flow for application: 10 ml/min
flow for elution: 4 ml/min
detection: 214 nm
column: HPLC steel column, 1 cm diameter, 12.5 filling height
column material: Source RPC 15 µm (Pharmacia, Freiburg)
equipment: BioCAD, Perseptive Biosystems, Wiesbaden-Nordenstadt

The eluate is collected in 4 ml fractions.

3. Mapping of the peptide fractions

3.1

Aliquots of the fractions obtained in 2.2 are applied to a Microbore reversed phase column and eluted in a gradient. Detection is effected with a UV detector and on-line with an electrospray mass spectrometer.

Chromatographic conditions:

flow for application: 20 µl/min
flow for elution: 20 µl/min
detection: 220 nm
column: C18 AQS, 3 µm, 120 A, 1 mm diameter, 10 cm length (YMC, Schermbeck)
equipment: ABI 140 B Dual Solvent Delivery System

buffer A: 0.06% trifluoroacetic acid in water
buffer B: 80% acetonitrile in A
gradient: 0% B to 100% B in 90 min

On-line mass spectrometry:

API III with electrospray interface (Perkin-Elmer, Weiterstadt)
positive ion mode
measuring range:m/z from 300 to 2390
scan time: 7 s
scan window: 0.25 m/z

Data acquisition is performed with MacSpec or MultiView Software (Perkin-Elmer).

3.2 MALDI MS measurement of the individual fractions

Aliquots of the fractions obtained in 2.2 are measured with different matrix substances, e.g., with the addition of L-(-)-fucose, in MALDI MS.

From the raw data, a multidimensional table is established considering the scan number, signal intensity and, after calculation, of the masses from the multiple-charged ions of a scan.

4. Comparative analysis

4.1 Identification of novel or lacking peptides or those significantly deviating in quantity

By comparing the data sets obtained under 3.3, which may also be referred to as peptide maps, qualitative and/or quantitative differences are established. Considering controls and samples, individual data sets or sets of data sets are used for comparison.

4.2 Peptide-chemical characterization of the identified targets

From the raw material obtained (e.g., large preparations of hemofiltrate), the identified targets are purified in such amounts as allow identification, using the different chromatographic separation techniques known to those skilled in the art which are generally employed for separating peptide mixtures (reversed phase, ion-exchange, size exclusion, hydrophobic interaction, etc.). After each chromatographic separation of a fraction, the targets are again identified in the fractions by ESI MS, MALDI MS or LC MS. This procedure is repeated, with variation of the chromatographic parameters, until a pure product of the desired specification, i.e., retention time and molecular weight, has been obtained. This is followed by the determination of a partial or complete amino acid sequence or a fragment pattern. Subsequently, a data base comparison is performed with the known data bases (Swiss-Prot and EMBL-Peptid- und Nucleinsäure-Datenbank), with the object to identify the partial or complete sequence or a fragment pattern. If no data base entry exists, the primary structure is clarified.

Example 2

Use of body fluids: ascitic fluid

1. Recovery of ascitic fluid

Ascitic fluid is formed as an extravascular exsudate in various diseases (malignant tumors, liver disorders etc.). According to the present method, between 10 ml and 10 l of ascitic fluid is obtained by punction and then immediately adjusted to a pH value of between 2.0 and 4.0 with diluted acid (e.g., 1 M HCl) in order to avoid proteolysis, and cooled to 4 °C. After ultrafiltration over a cellulose triacetate membrane with an exclusion size of 30 kDa (Sartocon mini-apparatus, Sartorius), the filtrate is further used as a source of peptides.

2. Recovery of the ascitic fluid peptides and separation into fractions

2.1 Peptide extraction with gradient elution

5 l of ascitic fluid filtrate is adjusted to pH 2.0 and separated through a preparative reversed phase column.

Chromatographic conditions:

flow for application: 40 ml/min
flow for elution: 40 ml/min
detection: 214 nm, 280 nm
column: Waters cartridge system, 4.7 cm diameter, 30 cm filling height
column material: Vydac RP-C18, 15-20 µm
equipment: BioCAD, Perseptive Biosystems, Wiesbaden-Nordenstadt
buffer A: 0.1% trifluoroacetic acid in water
buffer B: 80% acetonitrile in A
gradient: 0% B to 100% B in 3000 ml

The eluate is collected in 50 ml fractions.

The further course of the characterization corresponds to that in Example 1.

Example 3

Use of body fluids: urine

1. Recovery of urine

Urine is directly recovered as catheter urine or spontaneous urine from patients in amounts of from 0.5 to 50 l and immediately adjusted to a pH value of between 2.0 and 4.0 with diluted acid (e.g., 1 M HCl) in order to avoid proteolysis, and

cooled to 4 °C. After ultrafiltration over a cellulose triacetate membrane with an exclusion size of 30 kDa (Sartocon mini-apparatus, Sartorius), the filtrate is further used as a source of peptides.

2. Recovery of the urine peptides and separation into fractions

2.1 Peptide extraction with stepwise elution

10 l of urine filtrate is diluted with water to provide a conductivity of 6 mS/cm, and its pH value is adjusted to 2.7 with HCl. The urine filtrate is then applied to a chromatographic column. After binding of the peptides, the bound peptides are eluted with a saline gradient.

Chromatographic conditions:

flow for application: 100 ml/min
flow for elution: 30 ml/min
detection: 214 nm
column: Vantage (Amicon, Witten), 6 cm diameter x 7 cm filling height
column material: Merck Fraktogel TSK SP 650 M
equipment: BioCAD 250, Perseptive Biosystems, Wiesbaden-Nordenstadt

buffer A: 50 mM NaH_2PO_4, pH 3.0
buffer B: 1.5 M NaCl in A
gradient: 0% B to 100% B in 2000 ml

The eluate is collected in 10 pools of 200 ml each.

2.2 Second chromatographic separation

The fractions are separately subjected to chromatography through a reversed phase column.

Chromatographic conditions:

flow for application: 10 ml/min
flow for elution: 4 ml/min
detection: 214 nm
column: HPLC steel column, 1 cm diameter, 12.5 cm filling height
column material: Pharmacia Source RPC 15 µm
equipment: BioCAD, Perseptive Biosystems, Wiesbaden-Nordenstadt

buffer A: 0.1% trifluoroacetic acid in water
buffer B: 80% acetonitrile in A
gradient: 0% B to 100% B in 200 ml

The eluate is collected in 4 ml fractions.

The further course of the characterization corresponds to that in Example 1.

C L A I M S :

1. A method for detecting the condition of an organism through the measurement of peptides from a sample of said organism containing high- and low-molecular weight peptides, as an indication of the condition of said organism, wherein

   - low-molecular weight peptides are directly detected and characterized; and

   - related to a reference.

2. The method according to claim 1, wherein said sample is tissue or fluid samples from said organism, or the organism itself, or combinations thereof.

3. The method according to claims 1 and/or 2, wherein said low-molecular weight peptides used for said measurement have a molecular weight of not more than 30,000 Dalton.

4. The method according to claim 3, wherein said low-molecular weight peptides used for said measurement have a molecular weight which at least corresponds to that of dipeptides.

5. The method according to claims 3 and/or 4, wherein said low-molecular weight peptides used for said measurement have a molecular weight of from 100 to 10,000 Dalton.

6. The method according to at least one of claims 1 to 5, wherein said high-molecular weight peptides are separated off prior to measurement of said low-molecular weight pep-

tides, or left unconsidered, in terms of measurement or evaluation, in the recording of the sample.

7. The method according to at least one of claims 1 to 6, wherein the detection of said low-molecular weight peptides is effected by mass spectrometry.

8. The method according to at least one of claims 1 to 7, wherein said low-molecular weight peptides are characterized through the measurement of their molecular weights.

9. The method according to at least one of claims 1 to 8, wherein said sample is divided into different fractions prior to said measurement of the low-molecular weight peptides, and the fractions are measured under different conditions.

10. The method according to at least one of claims 1 to 9, wherein said organisms include procaryotes, eucaryotes, multicellular organisms, cells from tissue cultures, cells from animals and humans.

11. The method according to at least one of claims 1 to 10, wherein said sample is derived from genetically engineered or transformed and/or conditioned organisms.

12. The method according to at least one of claims 1 to 11, wherein the detection of the condition of the organism serves for examining and recording the overall condition of the organism without the need to recur to hypotheses in order to reveal any deviations from a reference condition.

13. The method according to at least one of claims 1 to 11, wherein the detection of the condition of a transformed organism serves for examining and recording the overall condition of the organism without the need to recur to hy-

potheses in order to reveal any changes of the transformed organism for revealing the occurrence of peptides connected with the transformation which are causally related to metabolic changes.

A b s t r a c t :

A method for detecting the condition of an organism through the measurement of peptides from a sample of said organism containing high- and low-molecular weight peptides, as an indication of the condition of said organism, wherein

- low-molecular weight peptides are directly detected and characterized; and

- related to a reference.

COMBINED DECLARATION FOR PAT (Includes Reference to PCT International Ap... 516 365 9805 ER OF ATTORNEY

ATTORNEY'S DOCKET NUMBER
Forssmann et al PCT

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

A METHOD FOR DETECTING THE CONDITION OF AN ORGANISM THROUGH THE MEASUREMENT OF PEPTIDES

the specification of which (check only one item below):

[ ] is attached hereto.

[ ] was filed as United States application

Serial No. _____

on _____,

and was amended

on _____ (if applicable).

[X] was filed as PCT international application

Number PCT/EP 97/04396

on August 13, 1997,

and was amended under PCT Article 19

on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
Germany	196 32 521.8	13 Aug. 1996	[X] YES [ ] NO
Germany	197 25 362.8	16 June 1997	[X] YES [ ] NO
			[ ] YES [ ] NO
			[ ] YES [ ] NO
			[ ] YES [ ] NO

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I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclose in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

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U.S. APPLICATIONS			STATUS (Check One)		
U.S. APPLICATION NUMBER	U.S. FILING DATE		PATENTED	PENDING	ABANDONED
PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO.	PCT FILING DATE	U.S. SERIAL NUMBERS ASSIGNED (if any)			

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(List name and registration numbers):*

ALLISON C. COLLARD, Registration No. 22,532;
EDWARD R. FREEDMAN, Registration No. 26,048;
ELIZABETH COLLARD RICHTER, Registration No. 35,103;
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201	SIGNATURE OF INVENTOR 202	
X Wolf Forssmann	X Peter Schulz-Knappe	
DATE X 22/02/1999	DATE X 15/02/1999	

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PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS		STATUS *(Check One)*		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED

PCT APPLICATIONS DESIGNATING THE U.S.

PCT APPLICATION NO.	PCT FILING DATE	U.S. SERIAL NUMBERS ASSIGNED *(if any)*			

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SIGNATURE OF INVENTOR 203	SIGNATURE OF INVENTOR 204	
DATE 17/02/199	DATE 23/02/1999	